1 **Silencing of sterol glycosyltransferases modulates the withanolide**

2 **biosynthesis and leads to compromised basal immunity of *Withania somnifera***

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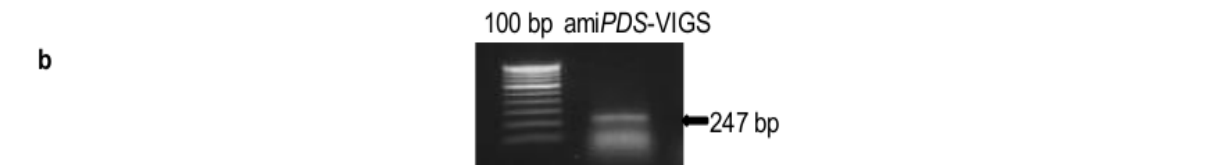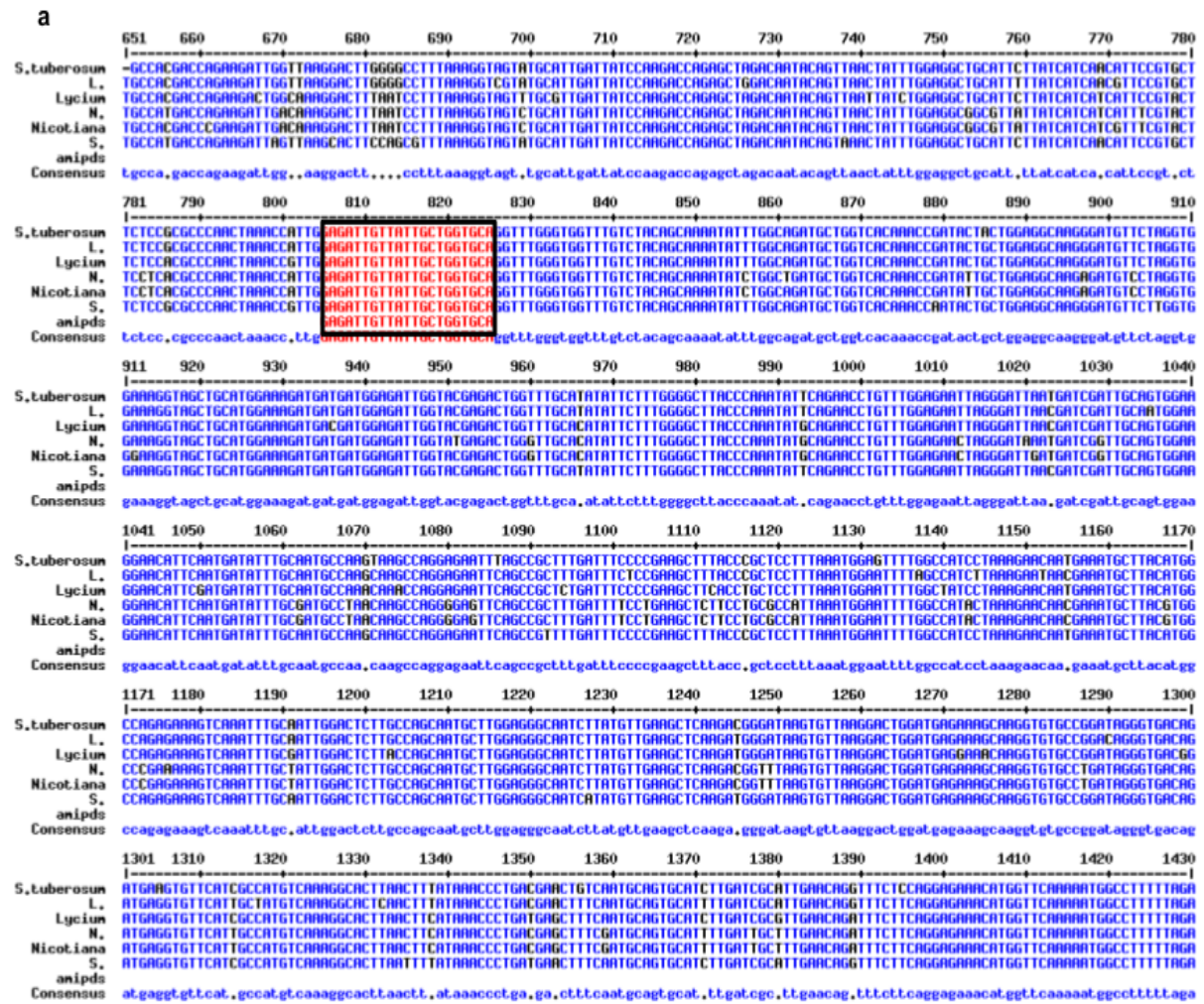8 [3]Banaras Hindu University, Varanasi, Uttar Pradesh, INDIA

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Supplementary Figure 1. Alignment of phytoene desaturase genes of Solanaceae and preparation of ami*pds*-VIGS construct.(a) Alignment of the phytoene desaturase gene of *N. benthamiana*, *S. nigrum*, *S. lycopersicum*, *L. esculentum*, *S. tuberosum*, through multialin online tool and 21 bp conserve region selected (red font black box) for preparation of primers. (b) Gel picture showed the cloned ami*pds* (247 bp) into VIGS vector.

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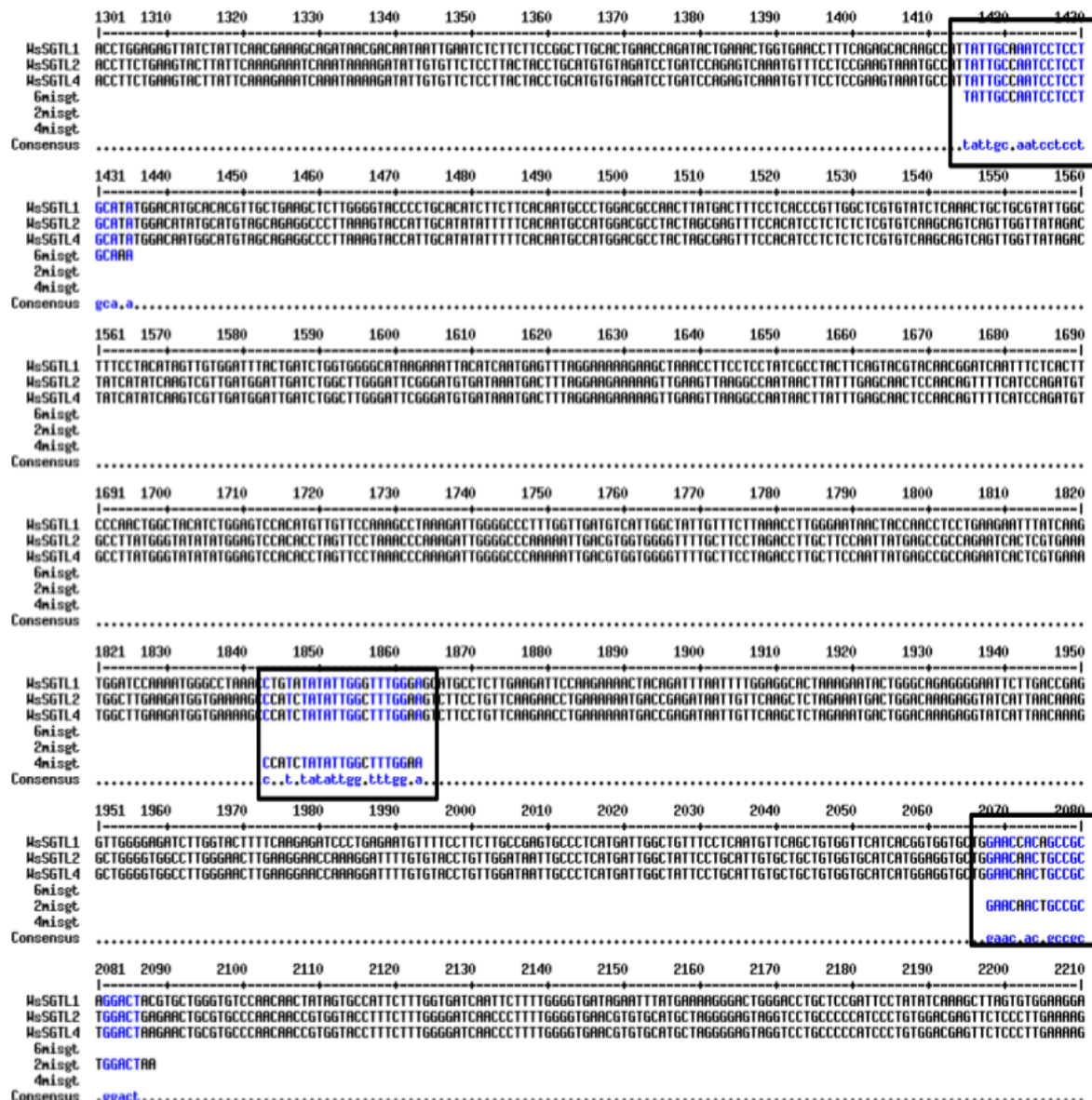
Supplementary Figure 2. amiRNA preparation for *WsSGTL* gene members of *W. somnifera*. Multiple alignments were performed by using of *SGTL1, SGTL2* and *SGTL4* of *W. somnifera* for the preparation of amiRNA through multalin tool. The highlighted regions showed the 21 bp sequence, selected for preparation of primers of amiRNA.

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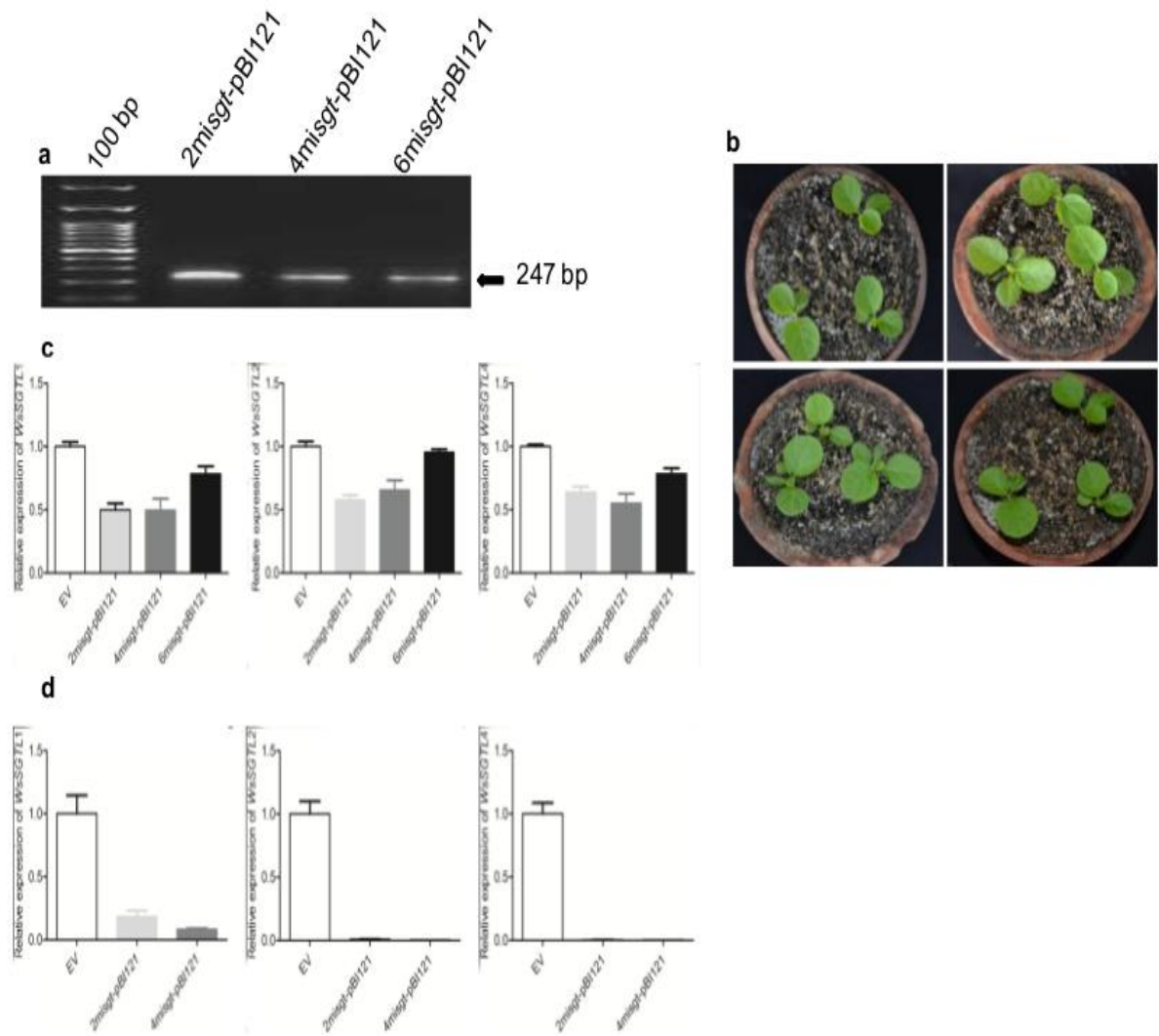
Supplementary Figure 3. Cloning and transformation of amiRNAs. (a) Gel picture of cloned 2mi*sgt* and 4mi*sgt* and 6mi*sgt* amiRNA into pBI121 vector (amplicon size 247 bp). (b) Stage for syringe infiltration in *Withania* i.e., 3 weeks old plants after seed germination. (c and d) qRT PCR of *WsSGTLs* members from the infiltered plant leaves by 2mi*sgt*-pBI121, 4mi*sgt*-pBI121 and 6mi*sgt*-pBI121 constructs after 48 h and 72 h.

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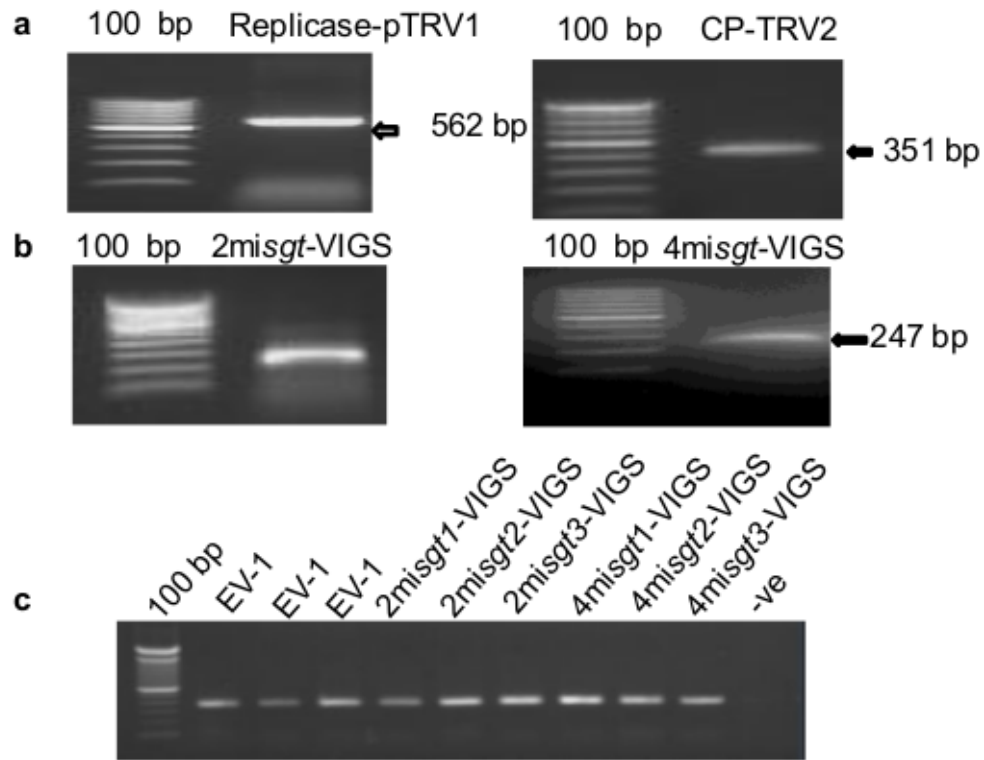
Supplementary Figure 4. aMIR-VIGS constructs of ami*sgt*s. (a and b) Colony PCR of positive VIGS constructs into GV 3101 strain of *Agrobacterium* by replicase, coat protein and by *WsSGTL* amiRNA specific primers. (c) Gel picture showed the confirmation of positive down-regulated lines of *W. somnifera* by coat protein specific primer after 3 weeks of the infiltration.

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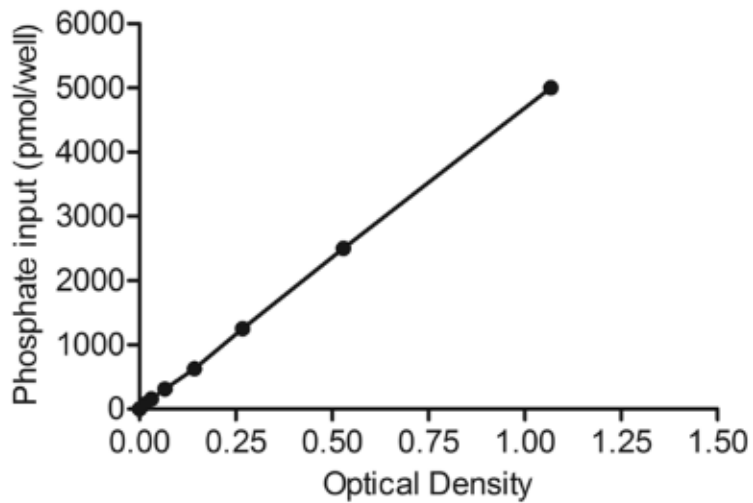
Supplementary Figure 5. **Standard curve preparation for SGT assay.** The slope

of the linear regression line, 4683 pmol/OD, represents the amount of phosphate

corresponding to a unit of absorbance at 620 nm.

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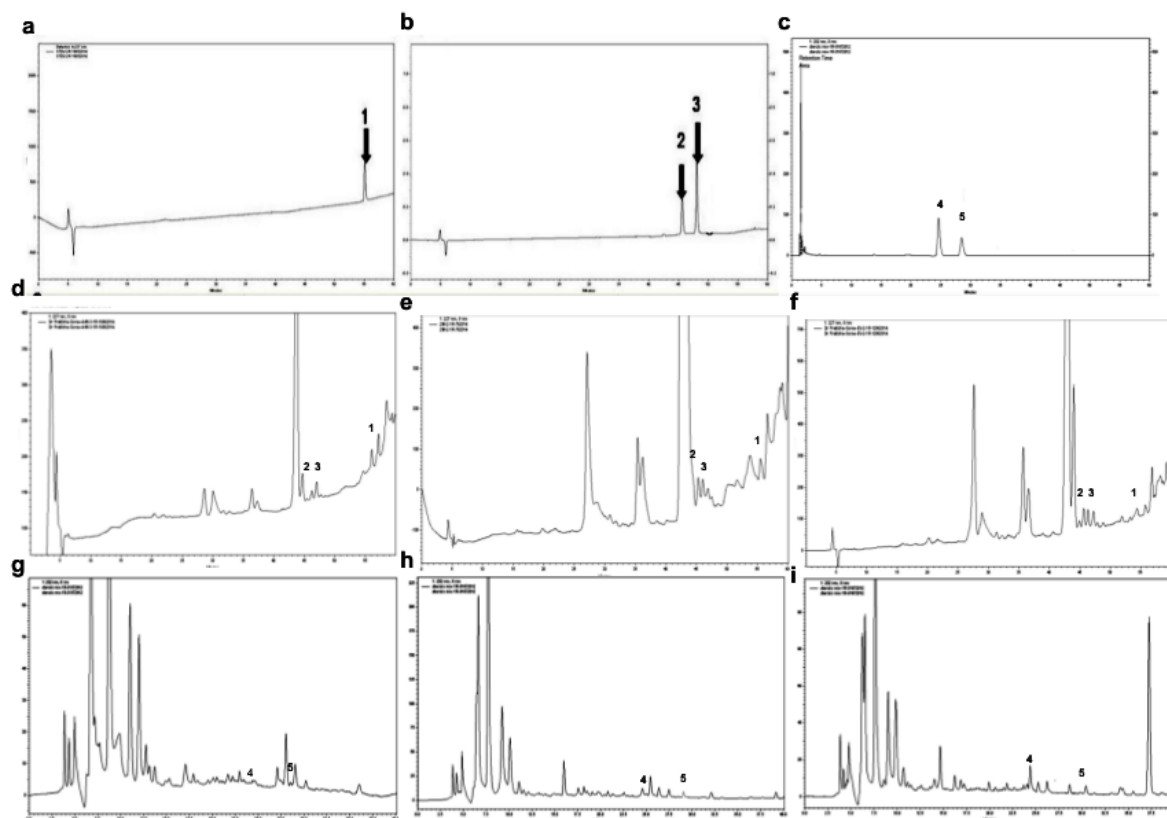
Supplementary Figure 6. HPLC chromatogram withanolides and sterols in EV and transgenic lines. (a, b and c) Standard HPLC chromatogram of 1) Withanoside V RT=55.12 (upper left), 2) withanolide A RT= 48.3 min, 3) withaferin A RT= 45.96 min (upper middle), 4) sitosterol RT= 28.48 and 5) stigmasterol RT= 24.6 (upper right). (d, e and f) HPLC Chromatogram of EV (central left), 2mi*sgt*-VIGS (central middle) and 4mi*sgt*-VIGS (central right) showing the relative abundance of different withanolides. (g, h and i) Chromatogram of the sitosterol and stigmasterol in the leaves of EV (lower left), 2mi*sgt*-VIGS (lower middle) and 4mi*sgt*-VIGS (lower right).

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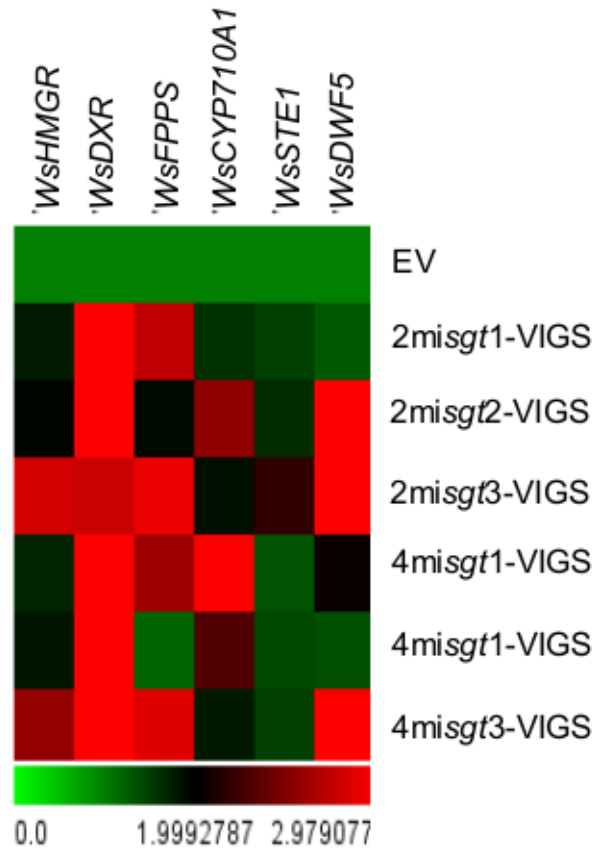
Supplementary Figure 7. Relative expression of important intermediate genes of withanolide biosynthesis pathway.Heat map showing the relative expression levels of MVA, MEP pathway genes in EV and leaves down-regulated lines. Data are means± SE of three biological replicate technical replicates using cDNA prepared from EV and transgenic plants. Expression levels of these genes were normalized to *Actin* and are represented in comparison with EV control

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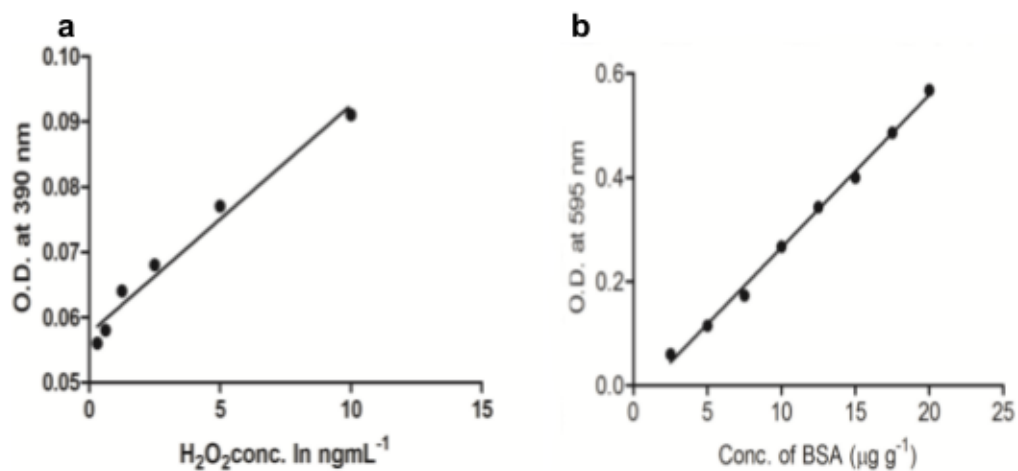
Supplementary Figure 8. Standard curve preparation for H$_2$O$_2$ and SOD. (a) Standard curve prepared by known concentration of hydrogen peroxide at 390 nm. (b) Standard curve preparation for Bradford assay by known concentration of bovine serum albumin (BSA) at 595 nm.

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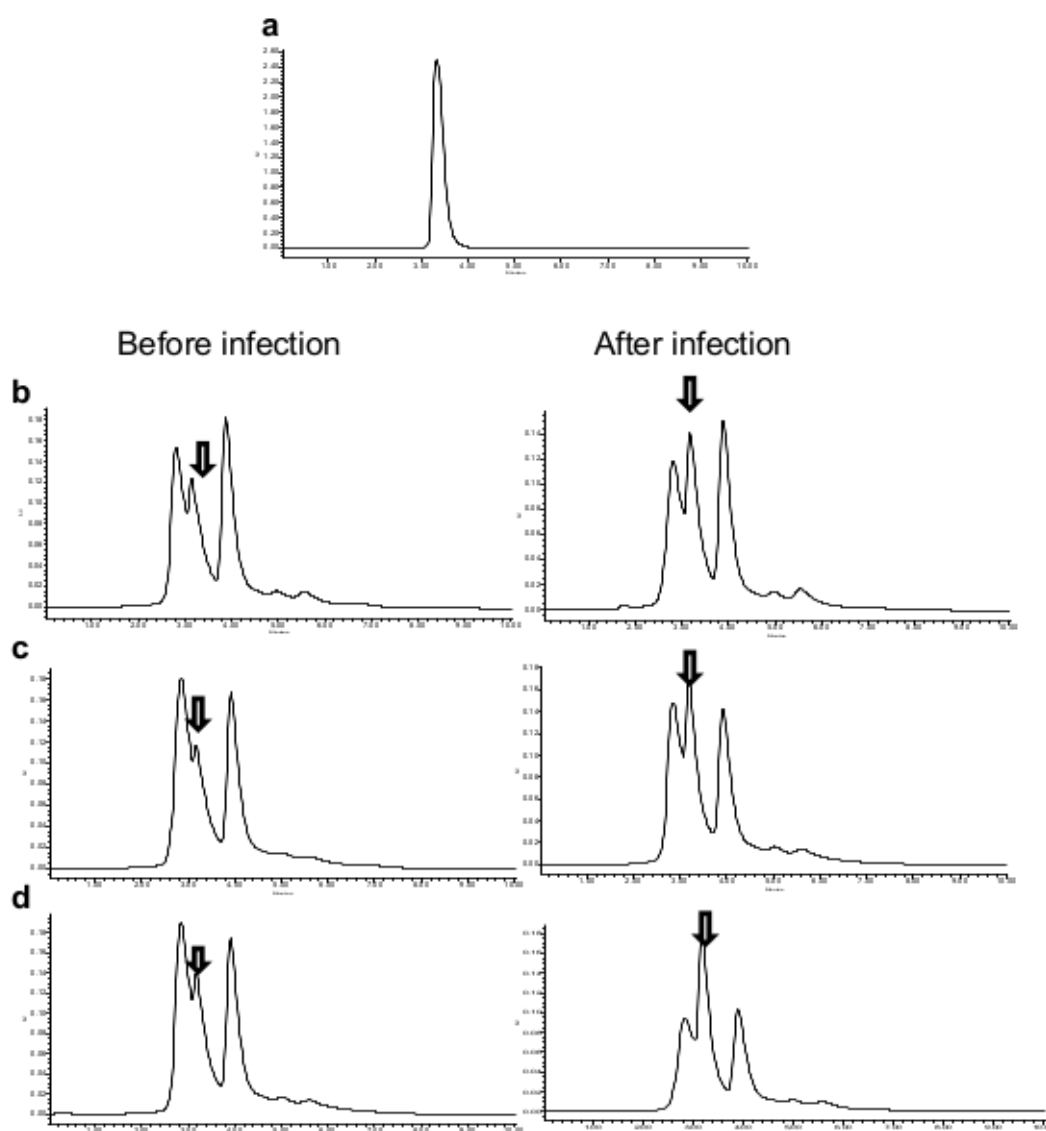
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Supplementary Figure 9. Salicylic acid estimation in EV and transgenic lines before and after infection of *A. alternata.* (a) HPLC chromatogram of salicylic acid (SA) standards at RT= 3.3. (b, c and d) The SA estimation in the leaves of EV and transgenic lines before and after 7 days infection of *A. alternata* of *W. somnifera* with photodiode array detection (PDA).

Supplementary Table 1. Designed amiRNA primers for the *PDS* and *WsSGTL* members.

Name	Sequences (5'-3')
ami*pds* F	AAGATAGATCTAGATCTGACGATGGAAGGAGATTGTTATTGCTGGTGCA CATGAGTTGAGCAGGGTA
ami*pds* R	AAAATAGAGCTCGTGAAAGAAGGAGATTGTTATTGCTGGTGCA AAAGAAGAGTAAAAGCCATTA
Ws*SGTL*2miF	AAGATAGATCTAGATCTGACGATGGAAGGAACAACTGCCGCTGGACTAACA TGAGTTGAGCAGGGTA
Ws*SGTL*2miR	AAAATAGAGCTCGTGAAAGAAGGAACAACTGCCGCTGGACTAAAAAGAA GAGTAAAAGCCATTA
Ws*SGTL*4miF	AAGATAGATCTAGATCTGACGATGGAAGCCATCTATATTGGCTTTGGAACA TGAGTTGAGCAGGGTA
Ws*SGTL*4miR	AAAATAGAGCTCGTGAAAGAAGCCATCTATATTGGCTTTGGAAAAAGAA GAGTAAAAGCCATTA
Ws*SGTL*6miF	AAGATAGATCTAGATCTGACGATGGAAGTATTGCCAATCCTCCTGCAAACA TGAGTTGAGCAGGGTA
Ws*SGTL*6miR	AAAATAGAGCTCGTGAAAGAAGTATTGCCAATCCTCCTGCAAAAAGAA GAGTAAAAGCCATTA

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116 **Supplementary Table 2.** Primers used in the validation of this study.

Primers	Sequences (5'-3')
CPP F **CPP R**	CTGACTTGATGGACGATTCTT TGTTCGCCTTGGTAGTAGTA
ReplicaseF **ReplicaseR**	CGGCACCCGATCTACAAGAA GCCCACTCATCTAGCCCAAA
RT- *PDS*F **RT- *PDS*R**	AGGTGGAAAGGTAGCTGCAT GGCTGAATTCTCCTGGCTTG
RT-*WsSGTL*F **RT-*WsSGTL1*R**	GCCGAGTGCCCTCATGATT GTTGGACACCCAGCACGTAGTC
RT-*WsSGTL2*F **RT-*WsSGTL2*R**	TGCAATTTGAAGTTCCGGGG AGGGTTGCACATCTCCTCTC
RT-*WsSGTL4*F **RT-*WsSGTL4*R**	GAACAACTGCCGCTGGACTA CCTAGCATGCACACGTTCAC
miR159-F1 **miR159-F1**	ATATCTCCTTCATAGCTCTAATG AAATAACACGCTAAACATTGCTTCG
RT-*Actin (Act)*F **RT-*Actin (Act)*R**	AGATATTCAGCCTCTTGTCTGTG ATTGAGCCTCATCACCAACATA
RT-*WsPR1*F **RT-*WsPR1*R**	TGATGAGAAGCAATGGTATGACTAT CGATCAGACATCAGTTGGAAGT
RT-*WsDFSN*F **RT-*WsDFSN*R**	TGCTGGTTTTTGCTACTGAGGCA CAGAAGCAACGGCGACGGAATC
RT-*WsSPl*F **RT-*WsSPl*F**	ATGCCCGTCAAATTCATTAAGTTT TCCTCCAGTCTCCAACAATCTA

RT-*WsPR10*F	AGTTGCTCATATAGAAGTCAAGTGT
RT-*WsPR10*R	TCCATCATAGTTCAATCTCCATTCA
RT-*WsHMGR* F	GGAGTAGCAGGGCCGTTGTT
RT-*WsHMGR* R	GCTTGTGCCGAACCTGACAC
RT-*WsDXR* F	CTCCGACCGCTTAGTAAATCCA
RT-*WsDXR* R	GCGAAGCATCGAGAGGAGTT
RT-*WsFPPS* F	TCCAAACTGCCTCTGGACAA
RT-*WsFPPS* R	GGCGATGGATAGGCAATGAG
RT-*WsCYP710A1*F	CGGTCACCCTTTTGGGAAA
RT-*WsCYP710A1*R	CGGCGGAGTTCCTTATGTTC
RT-*WsSTE1*F	ACAAGCCCTCCAGGAATATG
RT-*WsSTE1*R	GGCAGTGCCACACGTTATAG
RT-*WsDWF5*F	AGGACTGCCCAAGAAATCAT
RT-*WsDWF5*R	TTCCACTGATCACGGTTCAT

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